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Europäisches Patent Nr.

European Patent No.

Brevet européen n°

1257168

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Munich,  
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(19)



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(11)

EP 1 257 168 B1

(12)

## EUROPEAN PATENT SPECIFICATION

(45) Date of publication and mention  
of the grant of the patent:  
**02.02.2005 Bulletin 2005/05**

(51) Int Cl.7: **A01N 1/02, C12N 5/06,  
A61D 19/02**

(21) Application number: **00980267.9**

(86) International application number:  
**PCT/US2000/030155**

(22) Date of filing: **22.11.2000**

(87) International publication number:  
**WO 2001/037655 (31.05.2001 Gazette 2001/22)**

### (54) METHOD OF CRYOPRESERVING SELECTED SPERM CELLS

VERFAHREN ZUR KRYOKONSERVIERUNG VON SAMENZELLEN

PROCEDE DE CRYOPRESERVATION DE CELLULES SPERMATIQUES SELECTIONNEES

(84) Designated Contracting States:  
**AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU  
MC NL PT SE TR**

Designated Extension States:  
**AL LT LV MK RO SI**

(30) Priority: **24.11.1999 US 167423 P  
05.01.2000 US 478299**

(43) Date of publication of application:  
**20.11.2002 Bulletin 2002/47**

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**Description****FIELD OF THE INVENTION**

5 [0001] The invention relates to a method for freezing sperm selected for a particular characteristic, as well as to a frozen selected sperm sample and methods of using such a sample. The invention is particularly useful for preserving sex-selected sperm.

**BACKGROUND OF THE INVENTION**

10 [0002] Over half a century ago, artificial insemination was introduced in the United States as a commercial breeding tool for a variety of mammalian species. Although artificial insemination was initially limited to regions relatively close to the site of sperm collection, advances in the cryopreservation and storage of sperm have facilitated widespread distribution and commercialization of sperm intended for artificial insemination or in vitro fertilization.

15 [0003] Further improvements in mammalian sperm collection, selection, cryopreservation, storage, and handling techniques have enhanced the ability of breeders to produce animals having desired traits. For example, advances in selection of mammalian sperm based on slight differences in physical characteristics has made it possible to separate sperm based on sex-type, that is, to select for cells containing either the X or Y chromosome. This technique allows the breeder to manipulate the relative percentage of X- or Y-type sperm in a sample and thereby determine offspring sex. The ability to select sperm based on sex-type or any other desirable characteristic provides an important tool for accelerating genetic progress, increasing production efficiency, and achieving greater flexibility in livestock management. Full exploitation of this tool, however, depends on the ability to freeze and store selected sperm.

20 [0004] A variety of methods are available for selecting cells; however, the selection and subsequent processing of sperm presents unique challenges because sperm are incapable of DNA repair and because of sperm morphology. Each sperm has an acrosome overlying the head and a tail, which are important for fertility and which are relatively susceptible to physical injury. In addition, sperm fertility decreases with increasing time between collection and use. As most of the available selection methods involve physical stresses and take time, selected sperm are typically somewhat compromised compared to non-selected cells. Fertility may be further reduced if the selection technique involves significant dilution. It has been suggested that this "dilution effect" may be due to the loss of protective components in 25 seminal plasma.

30 [0005] Flow cytometry is a particularly efficient selection method that has been employed for sorting sperm by sex-type. However, sorted sperm are subject to stresses beyond those normally encountered in standard artificial insemination or in vitro fertilization protocols. In particular, flow cytometry is time consuming, and, because of the physical constraints of flow cytometers, sperm must be diluted for sorting to levels that are not optimal for storage (usually to 35 on the order of  $10^5$ - $10^6$ /ml). Furthermore, sorted sperm intended for artificial insemination must be concentrated so that conventional packaging and delivery equipment can be used. The need for a concentration step thus exposes already somewhat compromised sperm to additional physical stresses.

40 [0006] US Patent No. 4,474,875 discloses a method of controlling the sex of mammalian offspring by separating sperm into fractions having the desired sex characteristics and artificially inseminating the female to produce offspring of the desired sex. The sperm is separated by applying a buoyant force or forces to a mixture of sperm in nutrient media so that separation occurs according to density of the sperm. BR 9704313 also discloses a method for sexing mammalian sperm, which sperm may then be cryopreserved.

45 [0007] The freezing of sperm also invariably reduces fertility, motility, and/or viability, and, although techniques for freezing unselected sperm are well known, no technique for cryopreservation of selected sperm has been described.

**SUMMARY OF THE INVENTION**

[0008] The present invention provides a method of cryopreserving sperm that have been selected for a specific characteristic. In particular, the invention provides a method for the cryopreservation of sperm comprising:

- 50 (a) obtaining a sperm sample;  
 (b) adding an initial extender and selecting sperm by flow cytometry, wherein said adding and selecting are accomplished in any order;  
 (c) cooling said selected sperm sample;  
 55 (d) isolating sperm from said selected cool sperm sample to produce isolated cool sperm;  
 (e) adding final extender to said cool isolated sperm to produce a cool suspension of sperm; and  
 (f) freezing said suspension of sperm.